MONO- AND POLYRESISTANT TUBERCULOSIS AT TB FOCAL POINT, HELEN JOSEPH HOSPITAL

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DECLARATION

I, Sarah Alexandra van Blydenstein, declare that this research report is my own work. It is being submitted for examination in fulfilment for the degree Master of Medicine in Internal Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree at this or any other University.

Signed by:

Sarah Alexandra van Blydenstein

On this 3rd day of June 2015

ABSTRACT

Drug resistant (DR) tuberculosis (TB) is a rapidly emerging health problem in the Republic of South Africa (RSA). Multidrug resistant TB attracts a great deal of scientific attention not only worldwide but also in RSA, however, mono- and polyresistant TB is a relatively under studied disease entity. This study explored the demographics and characteristics of the types of mono- and polyresistant TB groups, and tried to determine if there were any associations between type of TB resistance and treatment outcome. The cohort studied consisted of 194 patients who had attended the Helen Joseph Hospital TB Focal Point. There were five major types of DR TB identified, including rifampicin (RIF) monoresistant (34%, 66 patients), isoniazid (INH) monoresistant (32.5%, 63 patients), INH polyresistant (20.1%, 39 patients), RIF polyresistant (4.6%, 9 patients) and phenotypically sensitive TB (8.9%, 17 patients).

A concerning figure of 86.6% of the DR TB cohort tested HIV positive, compared to the national population where 10.2% are reported HIV positive. The median CD_4 was 67 cells/mm^3, and 30.4% of the HIV positive patients had a CD_4 count of less than 50 cells/mm^3. Only 36.6% of the HIV positive patients were on antiretroviral therapy at time of DR TB diagnosis. Neither serum albumin nor haemoglobin were found to be significant markers for the prediction of outcome in DR TB, and no significant differences in DR TB type nor outcome were found when looking at age groups and gender. Although the numbers were small RIF monoresistant TB emerged as the most common type of DR TB (34%), and as such represents a clinical entity that should be considered separately from MDR TB. Unfortunately due to small sample sizes, no significant interpretation could be made regarding the various subgroups of DR TB and the genetic mutations to understand if there are differences in clinical presentation, mortality and morbidity predictors and outcome, and this is a field that requires further investigation.

DEDICATION

For the many who suffer from Tuberculosis

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LIST OF ABBREVIATIONS

AFB	Acid fast bacilli
ART	Anti-retroviral therapy
DNA	Deoxyribonucleic acid
DR	Drug resistant
DS	Drug sensitive
DST	Drug sensitivity testing
EMB	Ethambutol
g/L	Grams per litre
INH	Isoniazid
HAIN	GenoTypeMTBDR*plus* HAIN Lifescience
HIV	Human immunodeficiency virus
HJH	Helen Joseph Hospital
Kg	Kilograms
MDR	Multi drug resistant
NHLS	National Health Laboratory Services
No.	Number
PZA	Pyrazinamide
RSA	Republic of South Africa
RIF	Rifampicin
SD	Standard deviation
TB	*Mycobacterium tuberculosis*
UK	United Kingdom
USA	United States of America
XDR	Extensively drug resistant
Xpert	Xpert MTB/RIF® Test

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Chapter 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Tuberculosis (TB) is a disease caused by *Mycobacterium tuberculosis* that has plagued mankind since antiquity. Discovered by Robert Koch in 1882, TB has since been found globally (Seung et al., 2004). TB "remains the leading single cause of death due to an infectious agent throughout the world" (Ramaswamy et al., 2003). The burden of disease is prominent in Africa (Vashishtha, 2009), and together with the HIV epidemic, is wreaking havoc on the health of many (Chakroborty, 2011). Anti-tuberculosis drugs have been in use for over half a century, yet the mortality remains high (Cattamanchi et al., 2009). Contributing to this situation is the prolonged treatment period, with accompanying poor compliance, inadequate chemotherapy (either due to incorrect prescribing, inadequate drug doses, poor compliance, poor absorption of the drugs) emerging drug resistance, the HIV epidemic and poor social circumstances (Cox et al., 2007). Transmission from a patient with resistant TB plays a major role in spread and development of multi-drug resistant TB (MDR TB), whereas acquisition of resistance seems to be the more important factor in development of monoresistant TB (Van Rie et al., 2000).

Drug resistant TB (DR TB) is a growing concern, and in the Republic of South Africa (RSA), issues exist regarding diagnosis, treatment and prevention (Hafkin, Gammino, & Amon, 2010). Of concern, 20% of TB cases globally are drug resistant (DR) in some form, with 5.3% being MDR (Jagielski, Augustynowicz-Kopec, Zozio, Rastogi, & Zwolska, 2010). A WHO report from 2011 gave South African figures as 1.8% of new cases of TB are MDR TB, and 6.7% of retreatment cases are MDR TB (WHO, 2011).

1.2 Definition

Monoresistance in TB occurs when the organism is exposed to a single drug, either as monotherapy, or in a regimen that is inappropriate or failing, creating an effective single drug pressure (Sandman, Schluger, Davidow, & Bonk, 1999). Streptomycin was the first drug described to which TB became resistant, and this resulted from the use of streptomycin as monotherapy for TB (Gillespie, 2002).

Resistance can occur as either primary or a secondary, where primary drug resistance is found in a patient who has never received anti tuberculosis treatment, and secondary drug resistance is found in a patient either during or following treatment for previously sensitive TB. Primary drug resistance can be outbreak or non-outbreak related, and it is important to differentiate between the two, as outbreak associated primary drug resistance has a seemingly worse prognosis, as described in a case series from a hospital in Johannesburg , South Africa (Sacks, Pendle, Orlovic, Blumberg, & Constantinou, 1999). Primary drug resistance is becoming more common, and is a cause for concern, requiring drug sensitivity testing (DST) at diagnosis, rather than after treatment failure (Victor et al., 2007). Monoresistant TB is resistance to one of the first-line anti tuberculosis drugs (i.e., isoniazid, rifampicin, pyrazinamide, ethambutol, or streptomycin). Poly drug resistant TB is resistance to two or more of the first-line anti-tuberculosis drugs, but not both isoniazid and rifampicin.

Data gathered includes laboratory findings of haemoglobin and serum albumin. The value used as cut-offs in this study for haemoglobin is according to WHO, with severe anaemia defined as a serum haemoglobin of less than 8g/dL (World Health Organisation, 2011). The serum albumin cut-off of 2.0g/L was arbitrary.

1.3 Drugs and drug resistance

The two drugs used in both the intensive phase and continuation phase of treatment are rifampicin (RIF) and isoniazid (INH). Resistance to these drugs has implications for treatment and outcome, and it is thought that resistant TB has a poorer outcome than sensitive TB (Caminero, Sotgiu, Zumla, & Migliori, 2010).

RIF is an antibiotic that inhibits deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) polymerase. Resistance occurs due to a missense mutation in the rpoB region (Campbell et al., 2001). RIF affects slowly metabolizing organisms, and consequently sterilizes the sputum (Gillespie, 2002). RIF is the foundation of anti-tuberculosis therapy, in that regimens that do not contain this drug have to be administered for a longer period of time and often include injectable drugs on a daily basis, an uncomfortable regimen that may lead to decreased compliance. This is one of the reasons why RIF monoresistance is a clinical problem (Sandman et al., 1999).

The most important risk factor for RIF monoresistant, as with INH resistance, is previous exposure to anti-tuberculosis drugs (Kuaban, Bercion, Jifon, Cunin, & Blackett, 2000; Ridzon et al., 1998). Diarrhoea has also been reported to contribute to development of RIF resistance, by contributing to malabsorption (Ridzon et al., 1998). Rif monoresistance has been associated with co-infection with HIV. Sandman et al suggest that there is potential for drug interactions with anti-retroviral therapy (ART) and anti TB treatment, creating selective drug pressures (Sandman et al., 1999). However, there are as yet no reports of decreases in TB treatment efficacy when administered with ART. The particular interactions described are those between RIF and protease inhibitors, and RIF and non-nucleoside reverse-transcriptase inhibitors (McIlleron, Meintjes, Burman, & Maartens, 2007).

Mutations in the rpoB region are associated with RIF resistance (Chakroborty, 2011), although true RIF monoresistance has previously been documented as being relatively uncommon and is often used as a marker for MDR TB (Evans, Stead, Nicol, & Segal, 2009).

INH is an antibiotic with a number of mechanisms of action, the main and best understood of which is inhibition of mycolic acid synthesis. Other mechanisms include disruption of DNA, lipid, nicotinamide adenine dinucleotide (NAD) and carbohydrate metabolism (Zhang & Yew, 2009). The major risk factor for developing INH resistance appears to be previous treatment with anti-tuberculosis drugs (Cattamanchi et al., 2009) and (Fox et al., 2011), although, unlike RIF monoresistant, INH monoresistance does not seem to be induced by interactions with ART (Hoopes, Kammerer, Harrington, Ijaz, & Armstrong, 2008). Different mutations are known to confer differing levels of resistance, for example, katG confers high level resistance, whilst inhA confers only low level resistance (Gillespie, 2002). It is therefore important to know the mutation in the organism in order to predict the level of resistance.

Some studies have suggested that some INH resistance genes may weaken the virulence of the TB bacillus, possibly improving clinical outcome in those patients with INH monoresistant TB (Cattamanchi et al., 2009). However, another study, conducted in the Western Cape province of South Africa (RSA) showed no association between INH resistance and outcome (Jacobson et al., 2011), although it did suggest poorer outcome for both sensitive TB and INH resistant TB in RSA than in Europe and USA. The prevalence of INH monoresistance has been reported in some parts to be 7% such as the United States of America (Hoopes et al., 2008), 8-9% in the United Kingdom (Maguire et al., 2011) and 2.7% amongst both new and retreatment cases in RSA (Weyer, Brand, Lancaster, Levin, & van der Walt, 2007). The lower reported levels of INH resistance among TB isolates in RSA are perhaps as a result of poorer reporting due to limited diagnostic testing. INH monoresistance is a cause for concern as it has been implicated as an initial event in the development of multi-drug resistant TB

(Hazbon et al., 2006). Resistance associated with the Beijing genotype strain shows particular predilection for development of further drug resistance (Cox et al., 2007). The Beijing genotype strain has been associated with an increase in resistant TB in RSA (Johnson et al., 2010).

INH resistance has been associated with several mutations, including katG and inhA (Chakroborty, 2011). INH monoresistance is associated with different mutations from multi-drug resistance, in that monoresistance is associated with inhA mutations, and multi-drug resistance being more frequently associated with katG. The katG gene codes for catalase peroxidase, an enzyme involved in the conversion of the prodrug INH to an active form of the drug. The inhA gene codes for enoyl-ACP reductase, an enzyme needed in the synthesis of mycolic acid (Hazbon et al., 2006).

Several other mutations have been identified, but clinical significance remains to be proven. Furthermore, some INH resistant strains "do not contain mutations in any known gene targets for INH resistance" (Hazbon et al., 2006), suggesting that other mutations implicated in conferring INH resistance remain to be found (Ramaswamy et al., 2003).

Ethambutol (EMB) is another first line drug, and resistance to it is often associated with resistance to INH (A. Jain et al., 2008). This is associated with inhA mutations, where a mutation in the RNA polymerase gene confers INH and EMB resistance (Chakroborty, 2011). Another mutation conferring EMB resistance is embB (Amita Jain et al., 2008; A. Jain et al., 2008). In a study from the Western Cape, RSA, surveillance done on resistant mutants revealed 50% to be EMB resistant (Hoek, Schaaf, Gey van Pittius, van Helden, & Warren, 2009).

Pyrazinamide (PZA) is the final drug used in first line therapy. It is a pro-drug that functions best in an acidic environment. Monoresistance to PZA has been described in Quebec, Peru, Poland and the Western Cape, RSA, all with pncA mutations (Louw et al., 2006) and (Napiorkowska, Augustynowicz-

Kopec, & Zwolska, 2010). However, PZA resistance is usually associated with MDR TB, with some studies quoting 91% PZA resistant mutants to also be resistant to INH and RIF (Louw et al., 2006). In another study from the Western Cape, RSA, 53.5% of drug resistant isolates were found to also be PZA resistant (Hoek et al., 2009). This finding is similar to a national study in RSA which shows 52.1% resistant strains also resistant to PZA (Mphahlele et al., 2008). This is concerning as PZA is often relied upon in second line therapy. DST for PZA is not often carried out as it is technically challenging, as the acidic environment in which PZA optimally works inhibits bacterial growth. Also, the variety of mutations in the pncA gene makes a molecular based technique of resistance identification difficult. For these reasons, PZA resistance is not often tested and is possibly under-reported.

Conducting genotypic and/or phenotypic drug sensitivity testing (DST) on TB is important because it allows treatment to be tailored according to the sensitivity pattern, resulting in a better clinical response (Thomsen, Bauer, Lillebaek, & Glismann, 2000). DST at TB diagnosis would avoid amplification of resistance, a situation where resistant populations of the TB bacilli are selected as a result of poor drug pressure due to a regimen to which the TB is resistant (Cox et al., 2007). This is particularly important in areas where there is a high level of pre-existing DRTB, and is well demonstrated by a study conducted in Central Asia, where it was shown that 17% of patients with poly-resistant TB developed MDR TB, and 7% of patients with existing MDR TB developed further resistance to first line drugs through administration of standard first line treatment without initial DST (Cox et al., 2007).

1.4 Tests for resistance

The current gold standard for testing presence of TB and resistance of TB is a specimen culture. Two other molecular tests were used in this study population, the Xpert, and the HAIN.

Xpert MTB/RIF® Test (Xpert) is a test used to identify TB DNA. In a study conducted in Johannesburg, the sensitivity of the Xpert test was 86%, comparing favourably with smear microscopy (59%), and in HIV positive individuals, the sensitivity only dropped to 84% for the Xpert. The specificity was more than 97% (Scott et al., 2011). Xpert was introduced in the TB Focal Point at HJH in an experimental capacity on extra pulmonary fine needle aspirate specimens from July 2010, and HJH began performing Xpert on sputum specimens in March 2011. As such, not all the patients in the cohort had access to the test.

A molecular assay for the detection of Mycobacterium tuberculosis complex, GenoTypeMTBDR*plus* HAIN Lifescience (HAIN) was also used in this study population. The HAIN test identifies the rpoB genetic mutation for RIF resistance, and either the katG or the inhA genetic mutation for INH resistance. The sensitivity of the HAIN test is 98.1% for RIF resistance, and 90.2% for INH resistance, with specificities of 97.8% for RIF and 100% for INH resistance (Hillemann, Rusch-Gerdes, & Richter, 2007).

1.5 Epidemiology

TB is a major concern in RSA, with RSA placing third globally regarding overall TB burden, and a prevalence increasing by 400% (SANAC, 2012). In a study conducted in Swaziland, a country within South African borders, the rates of DR TB among culture positive patients for the period 2009 - 2010 have been reported as around 15% and 49% for new and previously treated patients respectively (Sanchez-Padilla et al., 2012). A study conducted amongst miners in the North West province of RSA between 2003-2005 showed a rate of 4.3% DR TB, of those, 10.2% had INH resistance, 5.6% polyresistant, and 84.4% MDR TB (Calver et al., 2010). In a survey from 2007, RSA, any type of DR TB occurred in 7.7% of patients, with INH monoresistance being found in 2.6% of new treatment cases, and 2.9% in retreatment cases. RIF monoresistant was found in 0.2% new cases and 0.8%

retreatment cases. Overall, monoresistant TB (either RIF, INH, streptomycin, EMB) was found in 5.1% of all cases (Weyer et al., 2007).

It is difficult to comment on the prevalence of mono- and polyresistant TB and outcome of treatment, in RSA, with more of the studies focusing on MDR TB. It would be interesting to know whether mono- and polyresistant TB have an effect on outcome, and what the risk factors associated with DR TB are.

Chapter 2: METHODOLOGY

2.1 Aims and objectives

It is the aim of this retrospective analysis to review and describe the database on mono- and polyresistant TB cases treated at TB Focal Point at Helen Joseph Hospital (HJH) which is a public hospital in Johannesburg, Gauteng province, RSA, and services many surrounding clinics and geographical areas. TB Focal point deals specifically with drug resistant TB and with difficult to treat TB. We seek to comment on the proportion of mono- and polyresistant TB, making associations with risk factors and resistance profiles, and looking at outcomes in these groups.

The primary objective of this study was to review and describe the cohort of patients with mono- and polyresistant TB from the database at TB Focal Point at HJH, for the period 1st March 2009 to 31st December 2011.

The secondary objectives were as follows:

2.1.1 To determine the proportion of patients with RIF monoresistance and describe the mutations present in this group

2.1.2 To determine the proportion of patients with INH monoresistance and describe the mutations present in this group

2.1.3 To determine the proportion of patients with poly-resistant TB

2.1.4 To look at the association between patient characteristics and treatment outcome

2.1.5 To describe treatment outcomes for the above groups

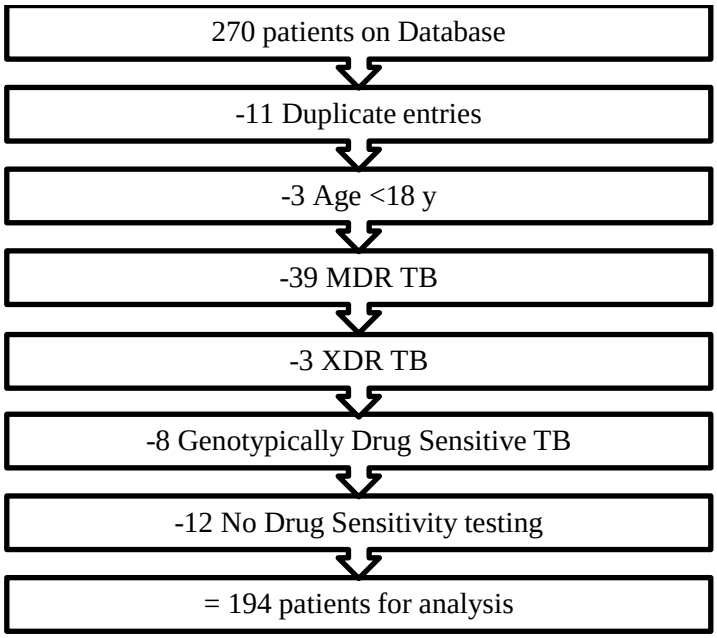
2.2 Study population

This study is a retrospective description of the database for the period 1st March 2009 to 31st December 2011, of patients who presented with DR TB to TB Focal Point. TB Focal Point is a specialised clinic dealing specifically with drug resistant TB. Patients could be diagnosed at TB Focal Point or were referred by local TB clinics after detection of DR TB. The database had 270 patients, and included data on demographics, laboratory specimens that confirmed the diagnosis, and information on treatment and outcome.

2.3 Data collection

Any patient in the database who had a result showing genotypic mono- or polyresistant TB was included. Conversely, in those with no results for either RIF or INH resistance, that patient's information was excluded. Duplicate entries were removed from the data, and patients less than 18 years of age were excluded. During the review of the database, 39 patients were found to have MDR TB, and were thus excluded. Similarly, three patients were found to have XDR TB and were excluded. Eight patients had genotypically drug sensitive (DS) TB and were excluded as they were on the traditional drug sensitive treatment regimen. Twelve patients had missing information about resistance and were excluded. This left 194 patients for analysis.

Chart 1. Patient Selection



The data was captured from the existing database using a data collection sheet (Appendix 1). Outstanding laboratory information was sought through National Health Laboratory Services (NHLS). Paper files held at TB Focal Point were assessed to update and complete the database for any missing clinical information.

Treatment outcomes could be: cured; treatment completed; died; failed; defaulted; or transferred out. The definitions of the treatment outcomes are based upon the national TB guidelines from RSA (Dept of Health, 2014). Cure for RIF monoresistant TB is defined as a patient who has completed treatment according to program protocol for 18 months after culture conversion and has at least five consecutive negative cultures from samples collected at least 30 days apart in the final 12 months of treatment. Cure for INH monoresistant TB is defined as a patient who has completed treatment according to program protocol for 6 months after culture conversion and has at least five consecutive negative cultures from samples collected at least 30 days apart in the final 12 months of treatment. Sputum conversion is defined as two negative sputum samples at least thirty days apart. If only one positive

culture is reported during that time, and there is no concomitant clinical evidence of deterioration, a patient may still be considered cured, provided that this positive culture is followed by a minimum of three consecutive negative cultures taken at least 30 days apart. Treatment will be considered to have failed if two or more of the five cultures recorded in the final 12 months of therapy are positive, or if any one of the final three cultures is positive (Dept of Health, 2014).

2.4 Data analysis and statistics

The analysis is based on 194 cases provided, and while not all cases had complete information, the analysis is based on the original sample.

All analysis was carried out using IBM SPSS version 21 software. X^2 and Fisher's exact test were used to compare proportions. Variables associated with DR TB were re-examined by a logistic regression model.

2.5 Ethical approval

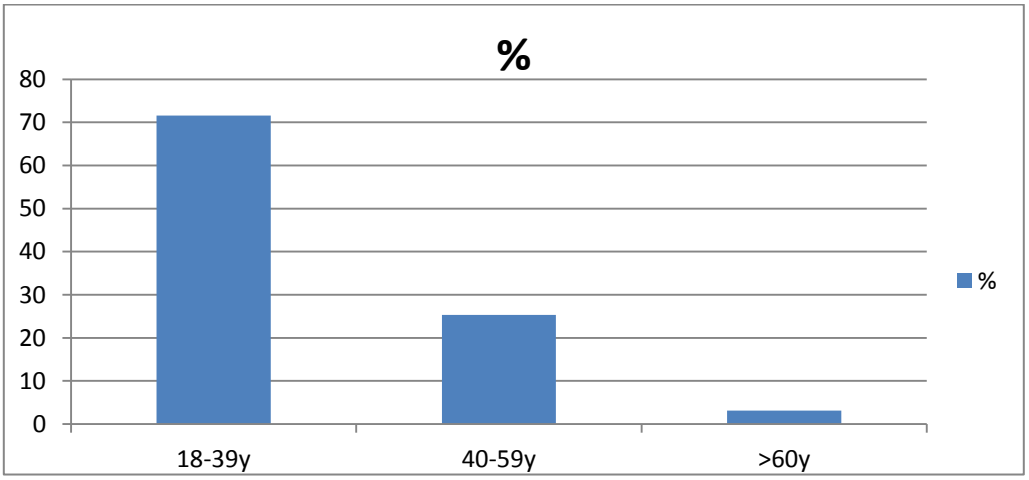
The research proposal has been approved by the WITS Human Research Ethics Committee, M120434.

Chapter 3: RESULTS

3.1 General characteristics of sample population:

The median age of the sample population was 34.5 years of age (SD ±10.0). Of the sample group, 71.6% of the patients were in the age group of 18-39; 25.3% of the patients in the age group 40-59; with 3.1% of the patients in the age group above 60 years of age. Sex of the patients in the sample was fairly even, with 49% male and 51% female. From the available data, 26% of the patients were in employment, while 40.2% of the patients were not in employment. A total of 130 cases were valid for analysis, representing 67.2% of the total dataset, leaving 33.8% unknown. No data on type of employment was included, as a precursor to the type of employment and predisposition to DR TB.

Graph 1: Graphical representation of age groups of the cohort



The median haemoglobin was 9.9 g/dL (SD ± 2.5). Data ranges from a minimum of 3.8 g/dL to 15.6 g/dL. A total of 120 cases were valid for analysis, representing 61.9% of the total dataset.

A haemoglobin of less than 8 g/dL was found in 14.4% of the cases, while 47.9% of the sample had a haemoglobin of more than 8 g/dL.

The serum albumin had a median of 2.2 g/L (SD ± 0.71). The range of albumin was minimum 1.2 g/L to maximum 4.2 g/L. A serum albumin of less than 2.0 g/L was found in 23.2% of the patients, while 36.1% of the patients had a serum albumin of more than 2.0 g/L.

A startling 86.6% of the patients were HIV positive; 8.8% of the patients HIV negative and in 4.6% of the patients the HIV status was unknown. A median CD_4 count of 63 cells/mm^3 was obtained for the sample, with a minimum value of 1 cells/mm^3 and a maximum value of 930 cells/mm^3. The lower quartile is 30.5 and upper quartile is 145.5, with an interquartile range of 115. A CD_4 count of less than 50 cells/mm^3 was found in 30.4% of the cases, while 26.3% of the patients a CD_4 count of 50-199 cells/mm^3, 9.3% a CD_4 count of 200-349 cells/mm^3; and 5.7% a CD_4 count of more than 350 cells/mm^3.

Of the patients who were HIV positive, 36.6% were on ART, 26.8% of the patients were not on ART and 36.6% of the patients had no data on whether or not they were on ART. A total of 194 cases were valid for interpretation.

The median weight at diagnosis was 54 kg (SD ± 10.0). A total of 131 cases were valid, representing 67.5% of the total dataset. The median weight at end of treatment was 62.2 kg (SD ± 10.5). A total of 89 cases were valid for analysis, representing 45.9% of the total dataset.

Chart 2: Percentage of major TB types

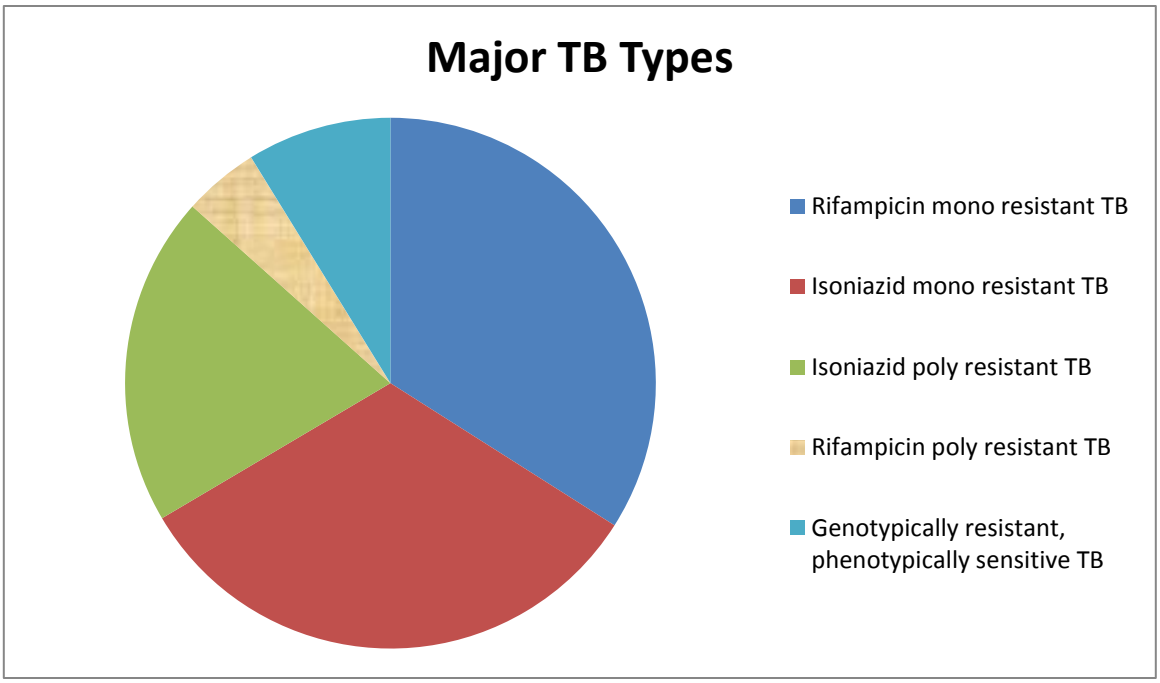


Table 1: Characteristics of sample population per DR TB group:

	Rifampicin monoresistant TB N = 66	**Isoniazid monoresistant TB N = 63**	**Isoniazid polyresistant TB N = 39**	**Rifampicin polyresistant TB N = 9**	**Phenotypically sensitive N = 17**
Age groups:	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p < 0.05$	$p > 0.05$
18 – 39 years	45/66 (68.2%)	47/63 (74.6%)	26/39 (66.7%)	8/9 (88.9%)	13/17 (76.5%)
40 – 59 years	21/66 (31.8%)	13/63 (20.6%)	10/39 (25.6%)	1/9 (11.1%)	4/17 (23.5%)
>60 years	0/66 (0%)	3/63 (4.8%)	3/39 (7.7%)	0/9 (0%)	0/17 (0%)
Sex:	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Male	31/66 (47%)	34/63 (54%)	18/39 (46.2%)	4/9 (44.4%)	8/17 (47%)
Female	35/66 (53%)	29/63 (46%)	21/39 (53.8%)	5/9 (55.6%)	9/17 (53%)
Employment:	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Yes	18/66 (27.3%)	15/63 (23.8%)	13/39 (33.3%)	3/9 (33.3%)	3/17 (17.6%)
No/unknown	48/66 (72.7%)	48/63 (76.2%)	20/39 (66.7%)	6/9 (66.7%)	14/17 (82.4%)
HIV status:	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Positive	59/66 (89.4%)	55/63 (87.3%)	31/39 (79.5%)	8/9 (88.9%)	15/17 (88.2%)
Negative	4/66 (6.1%)	5/63 (7.9%)	7/39 (17.9%)	1/9 (11.1%)	0/17 (0%)
Unknown	3/66 (4.5%)	3/63 (4.8%)	1/39 (2.6%)	0/9 (0%)	2/17 (11.8%)
Anti-retroviral therapy (of the patients with HIV):	N = 59	N = 55	N = 31	N = 8	N = 15
Yes	18/59 (30.3%)	19/55 (34.5%)	11/31 (35.4%)	3/8 (37.5%)	5/15 (33.3%)
No	41/59 (69.7%)	36/55 (65.5%)	20/31 (64.5%)	5/8 (62.5)	10/15 (66.7%)

The table below is a break-down per CD_4 class as specified in the dataset. The procedure was to analyse the major type of TB per CD_4 class within the dataset, and then an analysis was performed to understand if there is a significant difference between the classes and the types of TB. There were 137 patients with data available for CD_4 count.

Table 2: CD_4 class per TB type

Drug resistant TB Type	**No. of patients with CD_4 < 50 (%) n = 59/137**	**No. of patients with CD_4 50 – 199 (%) n = 51/137**	**No. of patients with CD_4 200 – 350 (%) n = 21/137**	**No. of patients with CD_4 > 350 (%) n = 11/137**
Isoniazid monoresistant	28 (20.4%)	16 (11.7%)	3 (2.2%)	2 (1.5%)
Isoniazid polyresistant	10 (7.3%)	15 (10.9%)	7 (5.1%)	3 (2.2%)
Rifampicin monoresistant	17 (12.4%)	17 (12.4%)	11 (8.0%)	6 (4.4%)
Rifampicin polyresistant	3 (2.2%)	3 (2.2%)	0 (0%)	0 (0%)
Genotypically resistant, phenotypically sensitive	1 (0.7%)	0 (0%)	0 (0%)	0 (0%)

Table 3: Type of specimen retrieved from patients

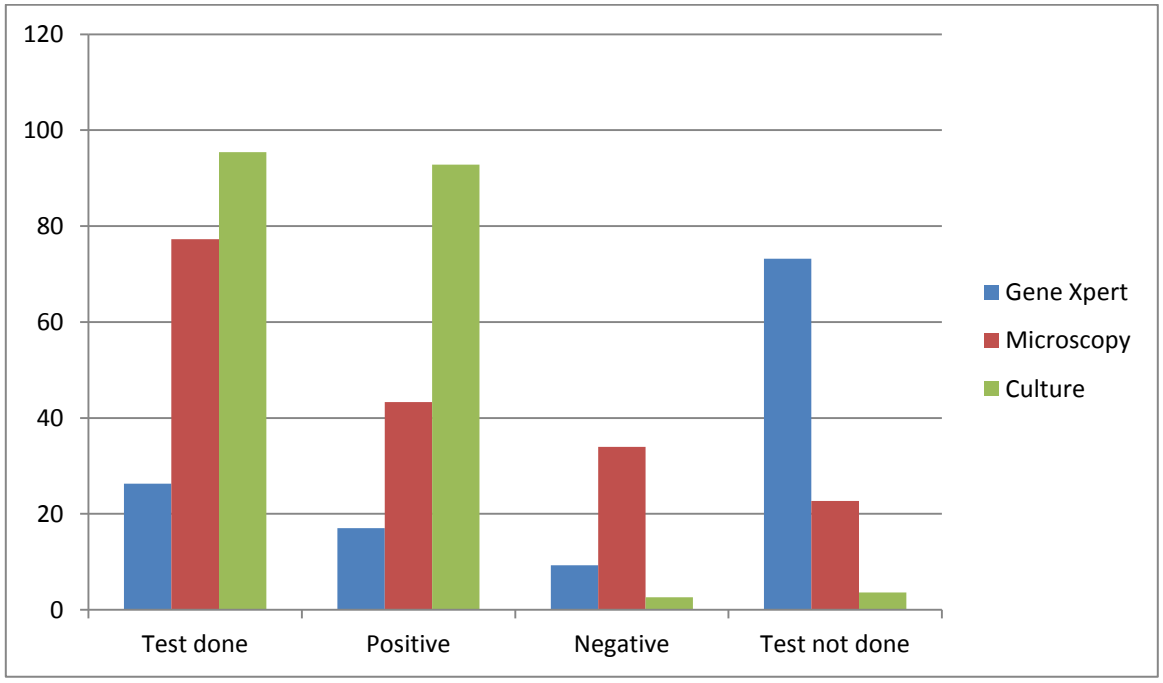
Type of Specimen	Percentage of patients with specimen retrieved (%)
Sputum	62.9
Blood	14.4
Fine needle aspirate	5.7
Fine needle aspirate and Sputum	3.1
Pus and Fine needle aspirate	2.6
Pleural fluid	2.1
Pus	1.5
Cerebro spinal fluid	1.5
Ascites	1
Blood, Pus and Fine needle aspirate	1
Blood and Ascites	1
Sputum, Fine needle aspirate and blood	1
Sputum and Bone Marrow	0.5
Blood and Cerebro spinal fluid	0.5
Sputum, Blood and Ascites	0.5
Sputum, Cerebro spinal fluid and Pleural fluid	0.5

A total number of 193 cases out of the 194 cases in the sample population were valid for analysis for Xpert MTB/RIF® Test (Xpert), representing 99.5% of the total sample. A positive Xpert result was found in17% of the patients, 9.3% of the patients had a negative Xpert, and 73.2% of the patients did not have an Xpert done or the result was unknown. Of the patients with an Xpert result, 22.2% of the patients had RIF Resistance. A total of 49 cases were valid for analysis, representing 25.3% of the total sample.

Initial sputum microscopy was performed on 77.3% of the patients, while 22.7% of the patients did not have a microscopy of the initial sputum sample done. A total of 194 cases were valid for analysis, representing 100% of the sample population. Of the patients who had microscopy performed, 43.3% had a positive microscopy for acid fast bacilli (AFB), while 34% of the patients had negative AFB microscopy. A total of 150 cases were valid for analysis, representing 77.3% of the total dataset.

Initial sputum culture was performed on 95.4% of the patients, while 3.6% did not have a culture done. Of those patients who had a culture performed, 92.8% of the patient's culture results indicated positive for TB, while 2.6% of the patient's culture results were negative for TB. The remainder had unknown results, and information regarding contamination of the specimen not being known.

Graph 2: Comparison of TB test performed



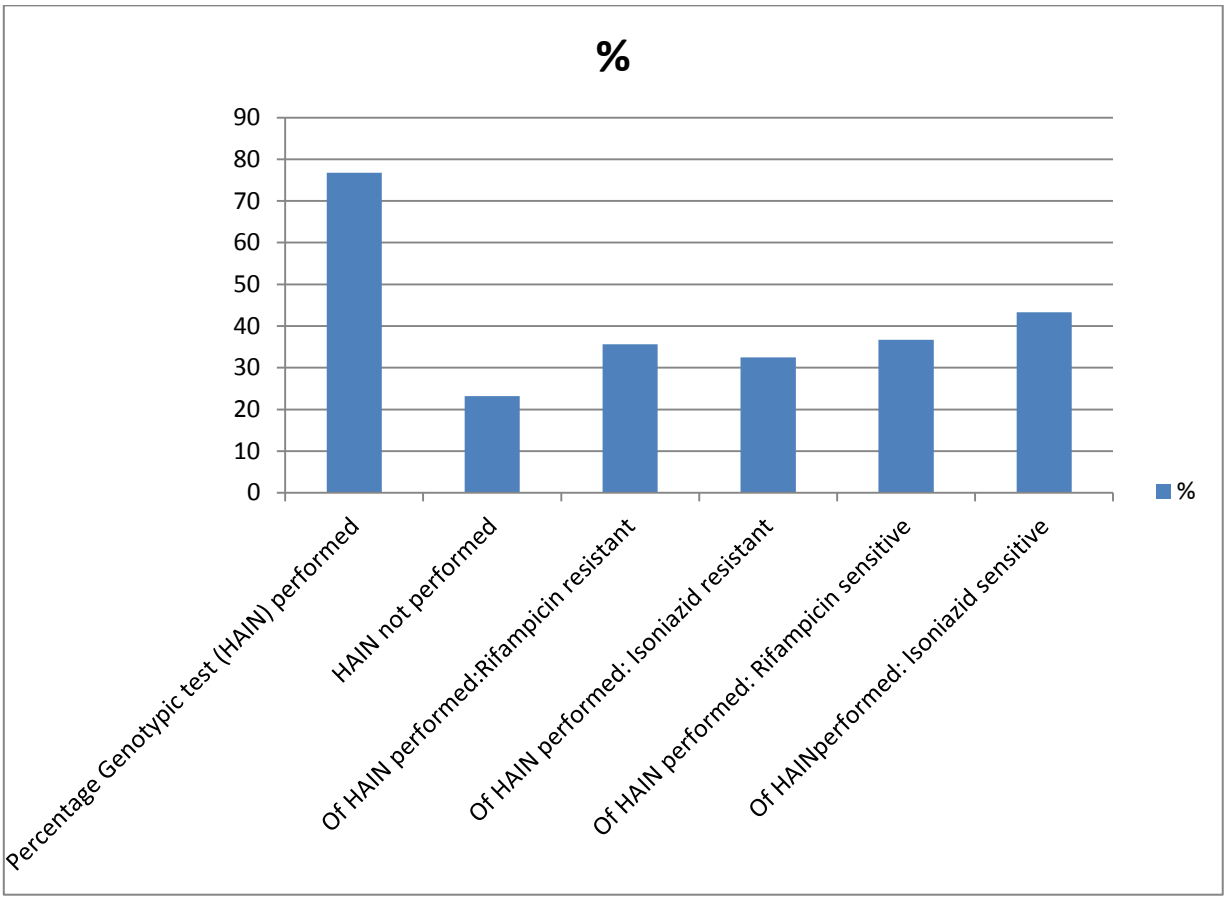
A molecular assay for the detection of Mycobacterium tuberculosis complex, GenoTypeMTBDR*plus* HAIN Lifescience, was performed on 76.8% of the patients, while 23.2% of patients did not have this test performed. At the time of the study, there was no protocol in place for the routine use of HAIN, and as such, specimens would undergo HAIN testing as an initial test if specifically requested by the treating doctor, otherwise, the HAIN was performed on culture positive specimens. Of all patients in which a genotypic drug sensitivity result was obtained, 43.3% of the patients were sensitive to INH;

32.5% of the patients were resistant to INH; and in 1% of the patients genotypic drug sensitivity testing to INH failed. No patients exhibited INH genotypic susceptibility testing as unknown.

Of all patients with a drug sensitivity result obtained by molecular testing, 37.6% of the patients exhibited sensitivity to RIF; 35.6% of patients resistant to RIF; 3.1% of patients unknown; and in 0.5% of patients drug sensitivity testing to RIF by the molecular assay failed.

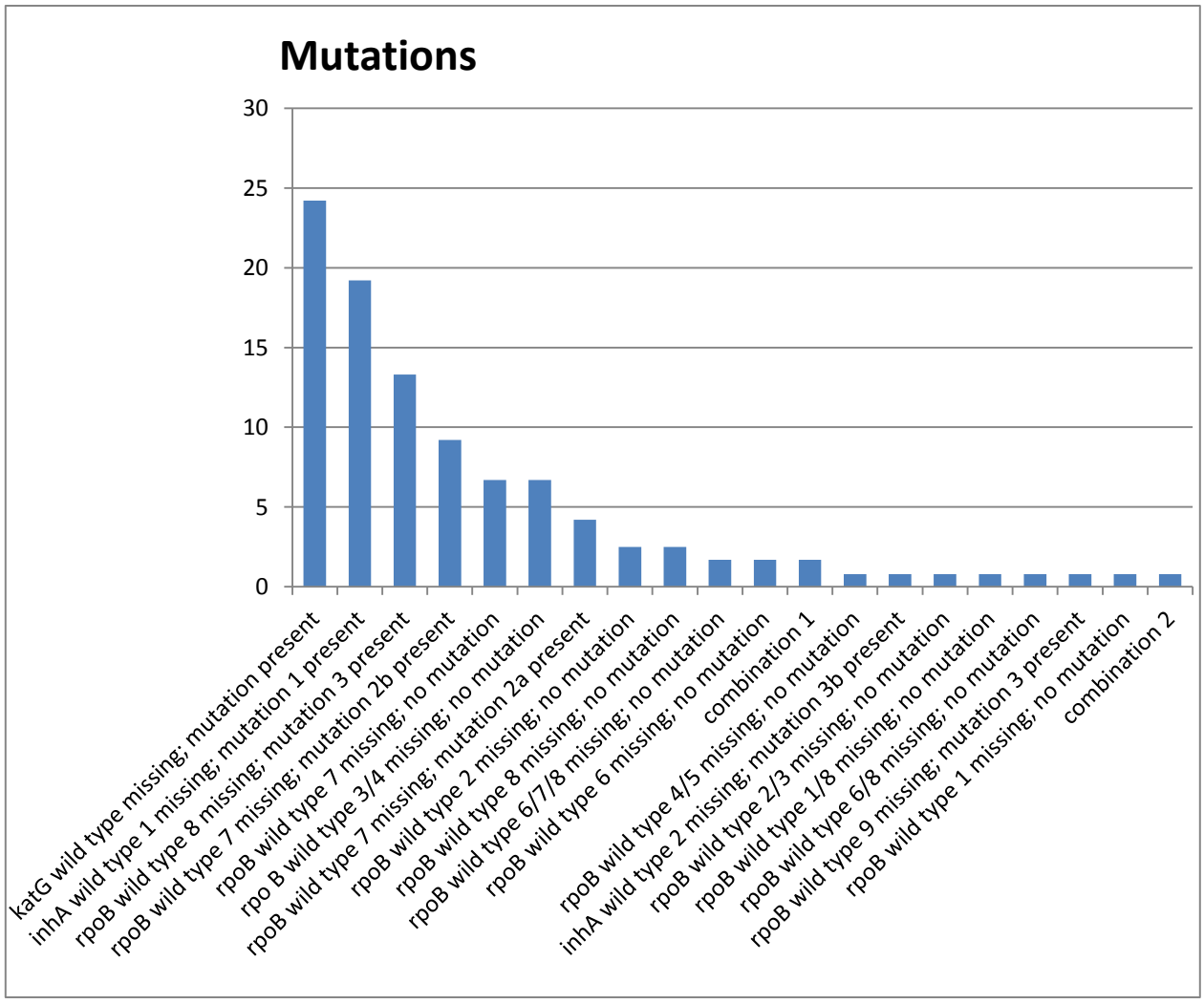
Graph 3: Graphical representation of genotypic drug susceptibility test results

Graph 3 indicates the results from the HAIN testing, where 76.8% of the samples underwent a HAIN test. Of those samples that underwent the test, the proportion of resistance is shown in the graph. The total adds up to more than 100% as some samples could have both INH and RIF resistance.



Genotypic susceptibility testing revealed a mutation in 61.9% of the patients, while for 38.1% the mutation was unknown. A phenotypic DST was conducted in73.7% of the patients, while 26.3% of the patients did not have a phenotypic DST done. A total of 194 cases representing 100% of the dataset were valid for analysis.

Chart 3: Percentage of mutation combinations in cohort



Combination 1: rpoB wild type 8 missing; no mutation, katG wild type missing, mutation 1 present, inhA wild type 2 missing; mutation 3b present

Combination 2: rpoB wild type 7 missing; no mutation, katG wild type missing; mutation present, inhA wild type 2 missing; mutation 3b present

3.2: RIF monoresistant TB

RIF monoresistant TB has the largest representation in terms of proportion of patients with TB .Of these patients, 4.5% were cured; 16.6% of the patients completed treatment; 9.1% of the patients died; 1.5% of the patients had treatment that failed; 13.6% of the patients defaulted on treatment; 9.1% of the patients were transferred out; 13.6% of the patients were still on treatment; 1.5% of patients were lost to follow-up; and 30.3% of patient's had files that were not retrievable.

Table 4: RIF monoresistant TB outcomes in various countries

Country, (total number cohort)	Cured, or treatment completed	Died	Lost to follow up, files not retrievable, transferred out	Relapsed	Still on treatment	Other (defaulted treatment, stopped,failed)
France (Meyssonnier, Bui, Veziris, Jarlier, & Robert, 2014) (30)	67% (n=20)	10% (n=3)	13% (n=4)	10% (n=3)		
Saudi Arabia (Singla, Al-Sharif, Al-Sayegh, Osman, & Shaikh, 2002) (18)	88% (n=16)			11.11% (n=2)		
South Africa, our findings (34)	21.2% (n=14)	9.1% (n=6)	40.9% (n=27)		13.6% (n=9)	15.1% (n=10)

If one removes the cases where the files were not retrievable, lost to follow up cases, and the cases that were transferred out, where the outcomes are not known, and are not necessarily poor, the new denominator total would be 39. In this case, the outcome for RIF monoresistance would be as follows.

Table 5: RIF monoresistant TB outcomes excluding cases with unknown outcomes

Outcome	Cured/treatment completed	Died	Failed treatment/defaulted	Still on treatment
Number of cases/39 (%)	14 (35.9)	6 (15.4)	10 (25.6)	9 (23.1)

Of the 34% of patients that had RIF monoresistant TB, 70.9% of these patients the mutations that were known, while 29.1% of these patients the mutations were not known.

Table 6: Mutations present and percentage in the RIF monoresistant TB group

Genetic mutation	Percentage of patients with mutation
rpoB 6/7/8 missing, no mutation, no katG mutation , inhA no gene locus	1.5
rpoB 6/8 missing, no mutation, no katG mutation, no inhA mutation	1.5
rpoB intense wild type 7, light wild type 8, no katG mutation, no inhA mutation	1.5
rpoB wild type 2 dark, wild type missing, with no mutations, no katG mutation, no inhA mutation	7.6
rpoB wild type 4/5 missing, no mutation, no katG mutation, no inhA mutation	3.0
rpoB wild type 6 missing, no mutation, no katG mutation, no inhA mutation	1.5
rpoB wild type 6/7/8 missing, no mutation, no katG mutation, no inhA mutation	1.5
rpoB wild type 7 missing, mutation 2B present, no katG mutation, no inhA mutation	3.0
rpoB wild type 7 missing, mutation 2A present, no katG mutation, no inhA mutation	13.6
rpoB wild type 7 missing, mutation 2B present, no katG mutation, no inhA mutation	3.0
rpoB wild type 7 missing, no mutation, no katG mutation, no inhA mutation	4.5
rpoB wild type 7 missing, no mutation, no katG mutation, inhA mutation	4.5
rpoB wild type 8 missing, mutation 3 present, no katg mutation, no inhA mutation	22.7
rpoB wild type 9 missing, mutation 3 present, no katG mutation, no inhA mutation	1.5
Unknown / not recorded	29.3

3.3: INH monoresistant TB

Isoniazid (INH) monoresistant TB had the second largest representation in terms of proportion of patients with DR TB. Of the 32.5% of patients that had INH monoresistant TB, 3.2% were cured; 20.6% of the patients completed treatment; 6.3% of the patients died; 6.3% of the patients had defaulted on treatment; 27.0% of the patients were transferred out; and 36.5% of the patients files were irretrievable.

Of the 32.5% of patients that had INH monoresistant TB, 47.7% of these patients the mutations that were known, while 52.3% of these patients the mutations were not known or not recorded.

Table 7: Mutations present and percentage in the INH monoresistant TB group

Genetic mutation	Percentage of patients with mutation
no rpoB mutation, katG wild type missing, mutation present, no inhA mutation	3.2
no rpoB mutation, katG wild type missing, mutation present, no inhA mutation	1.6
no rpoB mutation, no inhA mutation, katG wild type missing, mutation present	1.6
no rpoB mutation, no katG mutation, inhA wild type missing, mutation present	1.6
no rpoB mutation, no kat katG g mutation, inhA wild type1 missing, mutation present	12.7
no rpoB mutation, no katG mutation, inhA wild type 2 missing, mutation 3B present	1.6
no rpoB mutation, katG wild type missing, mutation present, no inhA mutation	22.2
rpoB wild type 2 missing, no mutation, no katG mutation, no inhA mutation	1.6
rpoB wild type 8 missing, mutation 3 present, no katG mutation, no inhA mutation	1.6
Unknown / not recorded	52.3

3.4: Polyresistant TB

There were 20.1% of the patients who had INH polyresistant TB; and 4.6% of the patients were RIF polyresistant TB. In the group of polyresistant TB, 3.6% had INH and EMB resistance; 2.6% had RIF and EMB resistance; 1.5% had RIF and streptomycin resistance; 0.5% had one of the following groups of resistance each: INH, streptomycin, EMB, ofloxacin, kanamycin; INH, EMB, ethionamide, streptomycin, PZA; INH, streptomycin, ethionamide; INH, streptomycin, PZA; RIF, ethionamide; or INH, EMB, ethionamide, and streptomycin resistance.

3.4.1 INH polyresistant TB

There were 20.1% (39 patients) with INH polyresistant TB. Of these patients, 7.7% were cured; 15.4% of patients completed treatment; 5.1% died; 35.9% of patients were transferred out; 2.6% of patients were still on treatment, 2.6% of patients were still on treatment; and 30.8% of patient's files were irretrievable. Mutations were known in 74.4%, while 25.6% had mutations that were not known or not recorded.

Table 8: Mutations present and percentage in the INH polyresistant TB group

Genetic mutation	Percentage of patients with mutation
No rpoB mutation, katG wild type missing, mutation present, No inhA mutation	2.6
No rpoB mutation, katG wild type missing, mutation present inhA wild type missing and mutation present	5.1
No rpoB mutation, no katG mutation, inhA wild type missing, mutation present	30.8
No rpoB mutation, katG wild type missing, mutation present No inhA mutation	25.6
rpoB wild type 2/3 missing, no mutations, no katG mutation, No inhA mutation	2.6
rpoB wild type 7 missing, no mutation, katG wild type missing, mutation present, inhA wild type 2 missing, mutation 3B present	2.6
rpoB wild type 7 dark wild type 8, no mutations katG wild type missing, mutation present, inhA wild type 2 missing, mutation 3B present	2.6
rpoB wild type 8 missing, no mutations katG wild type missing, mutation present, inhA wild type 2 missing and mutation 3B present	2.6
Unknown / not recorded	25.6

3.4.2: RIF polyresistant TB

There were 4.6% of patients that had RIF polyresistant TB, of which 11.1% completed treatment; 22.2% of patients had died; 44.4% of patients with RIF polyresistant TB defaulted on treatment; 11.1% of patients were transferred out; and 11.1% of patient's files were not retrievable.

In 66.7% of patients the mutations were known, whilst the remainder of the mutations were not known, or not recorded.

Table 9: Mutations present and percentage in the RIF polyresistant TB group

Genetic mutation	Percentage of patients with mutation
rpoB wild type 3/4 missing, no mutation, no katG mutation, No inhA mutation	11.1
rpoB wild type 6 missing, no mutation, no katG mutation, No inhA mutation	11.1
rpoB wild type7 missing, mutation 2A present, no katG mutation, No inhA mutation	11.1
rpoB wild type7 missing, mutation 2A present, no katG mutation, No inhA mutation	11.1
rpoB wild type 7 missing, mutation 2B present, no katG mutation, No inhA mutation	11.1
rpoB wild type 8 missing, mutation 3 present, no katG mutation and No inhA mutation	11.1
Unknown / not recorded	33.4

3.5: Genotypically resistant, phenotypically sensitive TB

These were patients who were Xpert RIF resistant, or resistant on testing with GenoTypeMTBDR*plus* HAIN Lifescience, but DST sensitive. These patients had been initiated on drug resistant TB therapy initially according to Xpert or HAIN results, but DST for first line TB drugs later proved phenotypically sensitive TB. The phenotypically sensitive group represented 8.8% (17) of all the patients. In this group, 5.9% of patients were cured, 23.5% of patients completed treatment, 5.9% of patients had treatment failed, 5.9% of patients had treatment defaulted, 41.2% were transferred out, 5.9% of patients were still on treatment, and 11.8% of patients files were not retrievable. Of the 8.8% of patients that were in the sensitive group, 53.1% of patients the mutations were known.

Table 10: Mutations present and percentage in the drug sensitive TB group

Genetic mutation	Percentage of patients with mutation
rpoB wild type 1 missing, no mutation, No inhA mutation, no katG mutation	5.9
rpoB wild type 1/8 missing, no mutation, no katG mutation, No inhA mutation	5.9
rpoB wild type 2 missing, no mutations, no katG mutation, No inhA mutation	11.8
rpoB wild type 2/3 missing, wild type 3/4 missing, no mutations, no katG mutation, No inhA mutation	5.9
rpoB wild type 3/4 missing, no mutation, no katG mutation, No inhA mutation	5.9
rpoB wild type 7 missing, no mutation, no katG mutation, No inhA mutation	11.8
rpoB wild type 8 missing, no mutation, no katG mutation, No inhA mutation	5.9
Unknown / not recorded	46.9

3.6 Treatment outcomes and factors predicting outcomes

Table 11: Treatment outcome of sample population

Treatment Outcome	% of patients n = 194
File not retrievable	58 (29.9%)
Transferred Out	45 (23.2%)
Treatment complete	35 (18%)
Treatment defaulted	18 (9.3%)
Died	14 (7.2%)
Still on treatment	11 (5.7%)
Cured	9 (4.6%)
Treatment failed	2 (1%)
Lost to follow-up or treatment stopped	1 (0.5%)

The below analysis is based on the CD_4 class and the type of outcome recorded.

Table 12: Representation of CD_4 class and treatment outcome

Treatment Outcome	No. of patients with CD_4 < 50 (%)	No. of patients with CD_4 50-199 (%)	No. of patients with CD_4 200-349 (%)	No. of patients with CD_4 > 349 (%)
Cure	1 (0.7)	3 (2.2)	1 (0.7)	1 (0.7)
Treatment completed	9 (6.6)	12 (8.8)	4 (2.9)	4 (2.9)
Died	6 (4.4)	5 (3.7)	1 (0.7)	0 (0)
Treatment failed	1 (0.7)	0 (0)	0 (0)	1 (0.7)
Treatment defaulted	6 (4.4)	1 (0.7)	4 (2.9)	0 (0)
Transferred out	9 (6.6)	11 (8.0)	4 (2.9)	2 (1.5)
Still on treatment	3 (2.2)	4 (2.9)	1 (0.7)	0 (0)
Lost to follow up	0 (0)	0 (0)	0 (0)	0 (0)
Treatment stopped	0 (0)	0 (0)	0 (0)	0 (0)
File not retrieved	24 (17.5)	15 (11.0)	3 (2.2)	3 (2.2)

The analysis below indicates the treatment outcomes per albumin class. A T-test to understand if significant differences occur between the groups in addition to tests of association that were computed to indicate if there is a practical output that can be used. The analysis is based on 115 valid cases for albumin.

Table 13: Representation of albumin class and treatment outcome

Treatment Outcome	No. of patients with Albumin < 2.0 g/L (%)	No. of patients with Albumin > 2.0 g/L (%)
Cure	1 (0.9)	5 (4.4)
Treatment completed	7 (6.1)	16 (13.9)
Died	7 (6.1)	4 (3.5)
Treatment failed	0 (0)	1 (0.9)
Treatment defaulted	4 (3.5)	6 (5.2)
Transferred out	7 (6.1)	11 (9.6)
Still on treatment	2 (1.7)	4 (3.5)
Lost to follow up	0 (0)	1 (0.9)
Treatment stopped	0 (0)	1 (0.9)
File not retrieved	17 (14.8)	21 (18.3)

No significant differences or associations were found between the 2 groups, thus indicating that albumin is not a predictor for treatment outcome ($P>0.05$).

The analysis below indicates the treatment outcomes per Haemoglobin class. A paired T-test to understand if significant differences occur between the groups as well as tests of association was computed to indicate if there is a practical output that can be used. The analysis is based on 120 valid cases for Haemoglobin.

Table 14: Representation of haemoglobin class and treatment outcome

Treatment Outcome	No. of patients with Haemoglobin < 8 g/dL (%)	No. of patients with Haemoglobin > 8 g/dL (%)
Cure	1 (0.8)	3 (2.5)
Treatment completed	6 (5)	17 (14.5)
Died	4 (3.3)	6 (5)
Treatment failed	0 (0)	1 (0.8)
Treatment defaulted	2 (1.7)	9 (7.5)
Transferred out	6 (5)	18 (15)
Still on treatment	1 (0.8)	5 (4.2)
Lost to follow up	0 (0)	1 (0.8)
Treatment stopped	0 (0)	1 (0.8)
File not retrieved	8 (6.7)	32 (26.7)

No significant differences or associations were found between the 2 groups, thus indicating that haemoglobin is not a predictor for treatment outcome.

In order to test the change in weight at the beginning and end of the treatment for significance, a T-test for one sample was computed at a 95% of confidence interval. This test indicates if there is a difference between the groups mean weights at the end and beginning of the treatment. The analysis was based on a sample size of 131 patients at the beginning and 89 at the end. A two-tailed test was performed as a difference in weight was being tested in either direction from the mean. The mean weight at the beginning was 54.6 kg, with a standard deviation of 10.1, while the weight at the end had a mean of 61.6 kg, with a standard deviation of 10.5. Statistically there is a significant difference ($p<0.05$) between the weights at the beginning and at the end of the treatment. There is thus an indication that

weight increases significantly after the treatment, however, ART remains a confounding factor. No data on date of initiation of ART was available, and as such, the increase in weight cannot solely be attributed to the treatment of TB.

Table 15: Representation of outcome per TB type

Outcome	**Rifampicin monoresistant TB %** **N = 66**	**Isoniazid monoresistant TB %** **N = 63**	**Rifampicin polyresistant TB %** **N = 9**	**Isoniazid polyresistant TB %** **N = 39**	**Genotypically resistant, phenotypically sensitive TB %** **N = 17**
Cure	4.5	3.1	0	7.7	5.8
Treatment Completed	16.6	20.6	11.1	15.9	23.5
Died	9.1	16.3	22.2	5.1	0
Treatment failed	1.5	0	0	0	5.8
Defaulted	13.6	6.3	44.4	0	5.8
Transferred out	9.1	27.0	11.1	35.9	41.7
Still on treatment	13.6	0	0	2.6	5.8
Lost to follow up	1.5	0	0	0	0
File not retrieved	30.3	36.5	11.1	30.7	11.8

Unfortunately, the majority of the cases had outcomes that were not quantifiable, in that they were not necessarily poor outcomes, (ie, death or treatment failure), but neither could they count as a good outcome (ie cure, treatment completed), making statistical analysis non-significant, as total numbers were too small.

Chapter 4: DISCUSSION

4.1 General overview

In a retrospective analysis we reviewed and described the cohort of patients with mono- and polyresistant TB treated at TB Focal Point at HJH. Our study population's mean age was older (34.5 years ± 10.7) than the rest of RSA (25 years) and Gauteng (28 years) (StatsSA, 2011). Our study found 71.6% of patients were between the ages of 18 years and 39 years, in keeping with many other studies showing that DR TB is more frequently found in the third and fourth decade (Coovadia, Mahomed, Pillay, Werner, & Mlisana, 2013; Dalton et al., 2012; Farazi et al., 2013; Hang et al., 2013). The younger age may be confounded by the relatively recent development of resistant strains, and spread of said strains.

One multicentre study, which included South African data, showed that in all countries they studied, DR-TB was more common in men than women (Dalton et al., 2012). There have been other differences found between sexes regarding DR TB, namely, fluoroquinolone resistance and XDR have been found more commonly in women than men (Dalton et al., 2012). Also, in a South African study it was shown that the ratio of confirmed TB cases was 2.1:1, female to male (Austin, Dick, & Zwarenstein, 2004). This was a study on TB, and not particularly DR TB, but does suggest that a gendered incidence of TB exists, however, no significant difference in gender was found in our study. The gender breakdown is what would be expected from a South African population, but may be representative of the catchment area around the HJH as well. This is in keeping with several other studies which showed no significant difference between sex and DR TB (Farazi et al., 2013; Kuaban et al., 2000).

The official unemployment rate in RSA was 25.2% for the first quarter of 2013. The expanded unemployment rate was 36,7% in the first quarter of 2013 – the highest since 2008. Our finding of

40.2% unemployed patients is in keeping with the South African statistics, but is considerably less than the 67.6% found in South African MDR-TB patients in a recent study (Dalton et al., 2012). A European (Faustini, Hall, & Perucci, 2006) study showed no increased risk of MDR TB with unemployment, and although our study was looking at mono- and polyresistant TB, our findings are in keeping with those of the European study.

Unfortunately, this study did not have access to data regarding the type of employment of those patients who were employed, as this might have shown a pattern of drug resistance. It has been shown in other studies that DR TB is increased in miners in RSA (Talbot et al., 2003).

The median haemoglobin concentration of 9.95 g/dL (SD ± 2.47) is higher than that found in a Mozambican study where the median haemoglobin concentration was 8.1 g/dL in DR TB patients, and 7.8 g/dL in DS TB patients (Nunes et al., 2005). 14.4% of the cohort had severe anaemia, a haemoglobin of less than 8g/dL. The difference is most likely not due to the resistance patterns of the TB as both MDR and sensitive TB patients had lower median haemoglobins than our patient cohort. The increased burden of malaria could possibly cause the observed lower haemoglobin in the Mozambican study.

The median serum albumin of the cohort was 2.2 g/L (SD ± 0.71), with 23.2% of patients having a serum albumin less than 2.0 g/L. Although the serum albumin did not correlate with any particular outcome nor the TB type in this study, the values demonstrated in this study are lower than those described in a Turkish study (Sahin & Yildiz, 2013). However, the Turkish cohort was not HIV positive, so a direct comparison is not reliable. Furthermore, our study does not have adequate data on comorbidities, for example malnutrition, chronic gastro enteritis, chronic inflammation, or liver problems, and, due to this confounding factor, we cannot draw any significant conclusions as to

whether serum albumin may be lower in DR TB than in DS TB. This is an area of possible future research.

Of the cohort, 86.6% were HIV positive, with a median CD_4 of 67 cells/mm^3 (SD 134.1), and 56.7% of cases with a CD_4 below 200 cells/mm^3. The low CD_4 count found is in keeping with Nunes et al (Nunes et al., 2005), who described a cohort of HIV positive patients, showing that DR TB predominantly had a CD_4 less than 100 cells/mm^3. There was no significant difference seen amongst the groups of patients regarding HIV reactivity, although the proportion of patients with HIV far outweighed those without HIV (86.6% of the patients were HIV positive and 8.8% were HIV negative). The presence of HIV did not predispose patients to a certain type of DR TB, nor predict an increase in a particular outcome, in keeping with various other studies from Botswana, Iran and RSA (Churchyard, Corbett, Kleinschmidt, Mulder, & De Cock, 2000; Farazi et al., 2013; Kuaban et al., 2000). However, the total number of patients who were HIV negative was small, and should significant inference like to be made regarding increased risk in patients with or without HIV, a larger sample of HIV negative patients would be needed. Only 36.6% of patients were on ART at the time of diagnosis of DR TB. There was no data regarding the percentage of patients started on ART after diagnosis of DR TB had been made following arrival at the TB Focal Point. This is in contrast to data from Malawi, reporting that 74% of the TB cohort studied in Lilongwe were co-infected with HIV, and 64% were on ART at the time of presentation (Vorkas et al., 2012). The high HIV prevalence found in this study (86.6%), accompanied by the low CD_4 on admission to the TB Focal Point, suggests advanced HIV disease, and this demonstrates a need to improve linkage to HIV care with better documentation of ART initiation.

In order to understand if there was a difference between the CD_4 count and the type of TB an independent T-Test was computed at a 95% confidence interval. While the numbers indicate that there

is a difference, statistically there is no difference ($p>0.05$) between the CD_4 count and the type of TB. This indicates that CD_4 count cannot be used as a predictor of the type of TB. These results may not be consistent with what is seen in practice, but based on the sample, CD_4 count is not a predictor (no associations) of type of DR TB.

A study conducted in Cameroon (Kuaban et al., 2000) showed that 59.6% of their patients had resistance to more than one drug, but their study included MDR patients, whereas our study excluded MDR and XDR TB. Our study found 24.7 % patients in the cohort had polyresistant TB, the difference possibly being accounted for by the exclusion of MDR patients.

Other studies have demonstrated repeatedly that the most significant risk factor for DR TB is prior treatment for TB (Dalton et al., 2012; Kuaban et al., 2000). Unfortunately, our database did not contain adequate information on previous treatment, and this parameter was not assessed.

Another variable we did not look at was geographical area. Dalton et al. found that resistance to second line injectable agents was more frequently found in Eastern Cape than in other provinces of RSA (Dalton et al., 2012). This is an important point to consider, as outbreaks could be localized, and having knowledge on local resistance patterns could help in suspecting and adequately managing DR TB patients prior to DST availability.

An interesting observation from our study was that the group with sensitive TB had a higher HIV positivity rate (88.2%) than the general population. A possible explanation being that HIV is a predisposition to TB. There were 35.3% of the sensitive TB group with HIV co-infection who were already on ART at the time of TB diagnosis, comparing favourably to the general population.

It was also noticed that the weight of patients increased with treatment of their DR TB, with a mean weight at the start of therapy of 54.5kg (SD 10.1kg) and at the end of treatment mean weight 61.6kg (SD 10.4kg). This is in keeping with recovery from a chronic illness.

This study demonstrated poor documentation of several factors, including type of therapy initiated, date of change of therapy, sputum testing on a monthly basis to follow up on treatment and cure rates, and there was poor archiving at this health facility. This suggests a need to improve documentation at patient visits, and a better archiving system. However, this study was conducted before the employment of a full time data capturer, which will hopefully resolve this issue. A suggestion that the health facility involves an infectious disease physician or a TB expert in the treatment of DR TB at the clinic level could also alleviate these problems.

4.2 RIF monoresistant TB

In studies conducted in Cameroon and Mozambique, it was found that RIF resistance was always associated with INH resistance, resulting in MDR TB (Kuaban et al., 2000; Nunes et al., 2005). In another study, conducted in Free State, South Africa, it was found that 2.5% (5/196) of DR TB patients had RIF monoresistance, with most RIF resistance occurring in the presence of MDR TB (Churchyard et al., 2000). RIF monoresistant TB was the most common TB type found in the study. Whilst RIF resistance has been regarded as a proxy for MDR TB, our study found that the largest group of resistance amongst our DR TB patients was RIF monoresistance, suggesting that the correlation between RIF monoresistance and MDR TB may not be as reliable as once thought. The increased frequency of RIF monoresistance has been reported by three other South African studies (Coovadia et al., 2013; Dramowski et al., 2012; Mukinda et al., 2012). In this study, we did not look at the correlation between genotypic and phenotypic testing regarding RIF monoresistance as a marker for MDR TB, however, as we report such a high number of RIF monoresistance, we suggest that

diagnoses of RIF resistant TB made by genotypic methods be confirmed by culture and sensitivity methods also, as outlined by the South African National Department of Health TB guidelines.

Our study found no significant difference between the sexes at a 95% significance level ($p>0.05$), in contrast to that described by Coovadia et al (Coovadia et al., 2013) reporting that males had a 42% increased odds of RIF monoresistant TB than females.

There is a significant difference ($p<0.05$) between patients with RIF monoresistant TB and HIV status, however caution should be heeded when interpreting that HIV positive patients are more prone to monoresistant TB, as the sample of HIV positive patients far exceeds the HIV negative patients, thus should an inference based on HIV status want to be made, a larger HIV negative sample is required. That said, there is still evidence that HIV patients are more susceptible to monoresistant TB. This is in keeping with Vietnamese data, reporting that HIV co-infection is a risk factor for RIF monoresistant TB (Hang et al., 2013). Of interest is that previous ART treatment may predispose to development of RIF monoresistant TB (Mukinda et al., 2012), and although this point was not addressed in the current study, it has important implications for the future.

Alcohol abuse has also been reported in RSA as a risk factor for RIF monoresistance (Mukinda et al., 2012), but was unfortunately not included in the data base the current study analysed.

The phenotypically sensitive group had rpoB mutations, or were missing the wild type, and had no katG nor inhA mutations. This group was genotypically resistant, but tested phenotypically sensitive on DST. It is possible that this group represents subclinical RIF resistance, and is an area of further research and attention.

Our data does not compare favourably with international data regarding cure or treatment completed for RIF monoresistant TB, however, the outcome may be affected by the large number of files not retrievable (30%) and by those patients still on treatment at the end of the study period (13%). Furthermore, 9% of patients were transferred to another care facility and no further information was traced.

4.3 INH monoresistant TB

INH monoresistant TB was found to be the second largest representation (32.5%) in terms of proportion of patients with resistant TB in our study. This was comparable to the 33% of patients with INH monoresistant TB in a study conducted in Cameroon (Kuaban et al., 2000), and the 29% in the cohort studied in Vietnam after excluding MDR (Hang et al., 2013). A study reported 15% INH monoresistance in Mozambique (Nunes et al., 2005) and a staggering 68.4% amongst DR-TB (this study included primary and acquired DR-TB) in a South African study conducted between the years 1993-1997 (Churchyard et al., 2000). The large number of patients with INH monoresistance seen in the Churchyard et al study could be accounted for by the study population, miners in Free State, RSA, residing in the mine compounds. These living conditions could predispose patients to outbreaks, similar to those observed in prisons and other settings where people live in close proximity to one another.

Another factor to consider is the inappropriate use of INH prophylaxis in patients who may have undetected or undiagnosed TB, resulting in mono-therapy and predisposition to develop resistance. Churchyard et al. did state that their study was conducted prior to the use of INH prophylaxis, and although not a factor in their study, remains an important consideration.

There was no significant difference between the sexes at 95% significance level, in keeping with many other studies on MDR TB (Kuaban et al., 2000).

There is a significant difference ($P<0.05$) between the age groups of patients with INH monoresistant TB, with the majority of patients falling into the 18-39 year group, however these difference, like RIF monoresistant TB, cannot be attributed solely to age and therefore interpretation as to the significant differences should be done cautiously.

Only 23.8% of the INH monoresistant patients were in employment, whilst the remainder were not in employment or employment was not recorded. Unfortunately, there was no data on type of employment, as a precursor to the predisposition of monoresistant TB. The unemployment rates of patients with INH monoresistant TB could be higher than in the general South African population, but the analysis is confounded by the number of patients with data missing or not known.

Most patients INH monoresistant TB (87.3%) were HIV positive, 7.9% of the patients were HIV negative, while 4.8% of the patients had a HIV status unknown. There is a significant difference ($P<0.05$) between patients with INH monoresistant TB and HIV status. Closely related to the HIV status, and the patients with INH monoresistant TB, is ART. Of the patients with INH monoresistant TB and HIV, 34.9% were on ART. More than half of patients with DR TB in RSA are co infected with HIV (Meintjes, 2014), compared to 4.6% of all TB patients in Israel who are co infected with HIV (Zohar, Moshe, Daniel, Noa, & Itamar, 2014), and a range varying from 0% (Slovakia and Slovenia) to 14.6% (Portugal) of co infection with HIV and TB (Pimpin et al., 2011). Controversy remains in the literature as to whether HIV infection is a risk factor for DR TB, with some studies in favour of the increased risk (Mesfin, Hailemariam, Biadgilign, & Kibret, 2014) and others not supporting the association (Berhan, Berhan, & Yizengaw, 2013). Of the reported 6,422,179 people living with HIV in South Africa, 31.2% are on ART as at October 2012 (Shisana et al., 2012). This compares with the proportion of patients with INH monoresistant TB and HIV co infection who are on ART at time of diagnosis of DR TB.

4.4 INH polyresistant TB

Kuaban et al showed that 12.2% of patients with DR TB had INH poly-resistant TB (Kuaban et al., 2000), comparable with Churchyard et al who showed 11.2% (Churchyard et al., 2000), and Nunes et al who showed 15% of patients with DR TB had INH polyresistance (Nunes et al., 2005). Our study found 20.1% of patients with INH poly, in keeping with a study from Burundi which described 22% of their DR TB being INH poly after excluding MDR TB (Faustini et al., 2006). This difference could be accounted for by the exclusion of MDR TB in our study regarding the Kuaban study, but Churchyard had 22/196 patients, and Nunes had 3/20 patients with INH poly and not MDR TB. Hang et al reported 39% of their cohort with DR TB as having INH polyresistance, after excluding MDR TB (Hang et al., 2013).

4.5 RIF polyresistant TB

Once MDR was excluded, RIF polyresistance was not described in a South African study (Churchyard et al., 2000), neither was RIF poly described in Mozambique (Nunes et al., 2005), nor in Vietnam (Hang et al., 2013). One patient in a European cohort of non MDR or XDR DR-TB was found to have RIF polyresistant TB (1.1%) (Faustini et al., 2006).

There is a significant difference ($P<0.05$) between the age groups of patients with RIF poly-resistant TB, however these difference cannot be attributed solely to age and therefore interpretation as to the significant differences should be done cautiously.

Chapter 5: STUDY LIMITATIONS

This study has several limitations.

At the time of the study, DR TB treatment in Gauteng was managed at various hospital levels, according to type of resistance. MDR TB was managed at a centralised hospital (Sizwe Hospital), while mono- and polyresistant TB were left to being managed at facilities like TB Focal Point, HJH. These facilities tasked with managing mono- and polyresistant TB did not have access to all drugs, did not have guidance on treatment or protocols, and there was no standardization of regimen or follow-up. This resulted in the non-uniformity of treatment, including drug choice and duration, making studies such as this one difficult to compare with other centers. Information regarding the initial drug treatment, and the potentially revised treatment after resistance result was not available to the study investigator.

The study only represents patients who presented to the health care facility, and does not consider the patients who have limited or no access to healthcare, and misses patients in whom the diagnosis of TB was missed. A panoply of reasons cause diagnostic delay and diagnostic failure including, health seeking delay on the patients part, provider failure to diagnose TB, care seeking between sectors and poor inter-sectorial communication, and use of the private sector. This can result in patients who should be treated in specialised care centres being missed, and thus excluded from adequate treatment, follow-up and possibly notification (Skordis-Worrall, Hanson, & Mills, 2010). A selection bias therefore exists within our study.

Given our small evaluable sample size, it was difficult to determine any associations between patient characteristics and drug-resistance or mortality.

Another limitation is the lack of complete data, resulting in a small sample size. This study was conducted before a full time clerk had been allocated to the database, and as such, the data is incomplete. Tracing old records and patient files is challenging as not all the information is recorded in a standardised fashion, and not all the information is available, either through lack of collection from the health care provider or from lack of adequate documentation. Data was updated and sought as far as possible, but some files were not found and as such, labelled missing.

Patients are down- referred to their local clinics for completion of DR TB treatment once they are stable, and are on oral treatment available at the local clinic and are stable. This was a limitation in the study as ethical approval had not been obtained to follow the patients up to their point of care at the local clinic; therefore many outcomes were missing in the study.

Many patients had results missing for follow up sputum collections. Laboratory reference error and tracking may have played a part, but also the down referral of patients made follow up of results difficult. This meant that in some cases, the diagnosis of cure could not be made, and the patient had to then be classified as treatment completed. Of concern, though, is that the sputum samples may not have been collected, representing a breakdown of provider care.

In this study, we did not have access to information regarding the patients past medical history regarding previous episodes of TB, and past treatment of TB, as well as information relating to adherence history. This is an important factor to consider, as previous treatment of TB was found as the only risk factor for acquired DR TB in a study from Cameroon (Kuaban et al., 2000).

We also did not gather information about alcohol use, tobacco use, imprisonment and unemployment, all of which are factors associated with increased risk for development of resistance to second line

injectable agents (Dalton et al., 2012) and looking for an association in first line treatment may have been fruitful.

Chapter 6: CONCLUSIONS

RIF monoresistant TB was the largest group in this study, highlighting the presence of RIF monoresistant TB as an entity separate to MDR TB. There is concern about the poor outcome of RIF monoresistant TB as 24.2% of patients in this study died or defaulted, compared to only 18.1% of patients who were cured or completed treatment. There were, however, also 13.6% of patients still on treatment at the time of the study completion. Our study showed no clear patient characteristics in terms of risk factors for acquisition of RIF monoresistant TB, nor any clear predictors of outcome. The most common mutations found in this group were rpoB wild type 8 missing, mutation 3 present (22.7%) and rpoB wild type 7 missing, mutation 2A present (13.6%). Unfortunately the numbers were too small to assess any differences between the mutations, but this would be an interesting field for further research. Given that one quarter of the patients had a bad outcome, RIF monoresistant TB needs further study to gain clarity on risk factors and factors affecting outcome in order to improve these outcomes.

Our study showed no significant risk factors for INH monoresistant TB, although most patients (87.3%), like the other DR TB groups, tested HIV positive. The common mutations found in INH monoresistant TB are katG wild type with a mutation (22.2%) and inhA wild type missing, mutation 1 present (12.7%). Proposed factors influencing INH monoresistant TB include occupation (especially mining in RSA), and possibly the prescribing of INH prophylaxis to HIV positive patients who are at risk for TB. Another potential risk factor could be outbreak related INH monoresistance. Our study did not address these factors but this remains an area for further research.

The mutations found most commonly in INH polyresistant TB were inhA wild type with mutation 1 present (30.8%), and katG wild type with mutation (25.6%). The proportion of INH polyresistant TB in our study was in keeping with other South African data.

In conclusion, RIF monoresistant TB was the most prevalent DR TB type in this cohort and treatment outcomes are poor. It is advised to treat any RIF resistant TB according to local MDR TB treatment guidelines. This study was unable to identify any predictors for DR TB type and DR TB treatment outcome, but research in this area should continue to enable the treating clinician to anticipate and aggressively manage patients with predicted poorer outcomes. The geographical pattern of sensitivity of TB is important as this could also guide clinicians in treatment selection, particularly in poorly resourced areas. Early appropriate treatment could lead to better control of the disease and limit spread of DR TB.

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Appendix ii

Data Collection Sheet

PATIENT CHARACTERISTICS								
Unique number:								
Age (years)								
Sex:	Male	Female						
Employed	Yes	No						
Laboratory results	Date:							
	Initial Haemoglobin value:							
	Date:							
	Initial Albumin value:							
HIV Status:	Positive	Negative	Unknown					
Baseline CD4 if HIV positive:								
ART if HIV positive:	Yes	No						
Co-Morbid Disease	Yes	No						
If Yes, describe								
TB DIAGNOSIS								
Type of Specimen:	Sputum	Blood	Pus	FNA	Ascitic fluid	Pleural fluid	CSF	Bone Marrow
GeneXpert MTB:	Positive	Negative	Unknown / Not done					
Rifampicin Resistance detected on GeneXpert:	Yes	No						
Microscopy done:	Yes	No						
If Microscopy done	AFB +	AFB -						
Culture done:	Yes	No						
If culture done, result	Positive	Negative						
PCR result on Culture	MTB	No MTB						
HAIN PCR Done	Yes	No						
HAIN PCR INH	Sensitive	Resistant	Inconclusive	Failed				
HAIN PCR RIF	Sensitive	Resistant	Inconclusive	Failed				
Resistance mutation known:	Yes	No						
If Mutation known, describe								
DST done:	Yes	No						

If DST done:	Sensitive:	Resistant:		Sensitive:	Resistant:
INH			Oflox		
Rif			PZA		
Strep			Amik		
Etham			Thiacetazone		
Ethio			Kana		

TB TREATMENT								
Initial treatment start date								
Weight at start of treatment (kg)								
Weight at end of treatment (kg)								
Initial Treatment:								
Rif	INH	PZA	EMB	Ethio	Amik	Strep	Oflox	Cipro
After Results:								
Date Changed:								
Rif	INH	PZA	EMB	Ethio	Strep	Oflox	Amik	Azith	Cipro
If initially sputum culture positive, document follow-up culture results								

Date sputum 1		**Date Sputum 2**		**Date Sputum 3**		**Date Sputum 4**	
Microscopy done	Yes	No					
Result microscopy	AFB positive	AFB negative					
Culture done	Yes	No					
Culture result	Pos	Neg					
PCR result on culture	MTB	No MTB					

Treatment Outcome and Date	Cure	Treatment completed	Died	Treatment failed	Treatment defaulted	Transfer out	Lost to follow up	Treatment stopped	File missing

Key:

Amik	Amikacin
ART	Anti Retroviral Treatment
Azith	Azithromycin
Cipro	Ciprofloxacin
CSF	Cerebro Spinal Fluid
DST	Drug Sensitivity Test
EMB	Ethambutol
Ethio	Ethionamide
FNA	Fine needle Aspirate
INH	Isoniazid
MDR	multi Drug Resistance
MTB	Mycobacterium Tuberculosis
Oflox	Ofloxacin
PZA	Pyrazinamide
Rif	Rifampicin
Strep	Streptomycin
TF	Transfer
XDR	Extensive Drug Resistance

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Sarah A van Blydenstein

CLEARANCE CERTIFICATE	**M120434**
PROJECT	Mono-and Polyresistant Tuberculosis Diagnosed at TB Focal Point, Helen Joseph Hospital (Revised title)
INVESTIGATORS	Dr Sarah A van Blydenstein.
DEPARTMENT	Clinical HIV Research Unit/Internal Medicine
DATE CONSIDERED	04/05/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 31/05/2012 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr E Jong

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

Gauteng Department of Health

Helen Joseph Hospital

PERMISSION FOR RESEARCH

DATE: 06/08/2012

NAME OF RESEARCH WORKER: DR SA VAN BLYDENSTEIN

CONTACT DETAILS OF RESEARCHER (INCLUDE ALTERNATE RESEARHER):

CELL: 071 893 7056

TITLE OF RESEARCH PROJECT MDHO & PDLY RESISTANT TB AT JB FOCAL POINT – HELEN JOSEPH HOSPITAL

OBJECTIVES OF STUDY (Briefly or include a protocol):

SEE ATTACHED PROTOCOL

METHODOLOGY (Briefly or include a protocol):

SEE ATTACHED PROTOCOL

CONFIDENTIALITY OF PATIENTS MAINTAINED: YES

COSTS TO THE HOSPITAL: NIL

APPROVAL OF HEAD OF DEPARTMENT: YES

APPROVAL OF CRHS OF WITS UNIVERSITY: YES

SUPERINTENDENT PERMISSION:

Signature: _____ Date: 06/08/2012

GAUTENG PROVINCIAL GOVERNMENT
HELEN JOSEPH HOSPITAL
P/BAG X47
06 AUG 2012
2006 JHB
HELEN JOSEPH HOSPITAL
GAUTENG PROVINCIAL GOVERNMENT

health and social development
Department: Health and Social Development
GAUTENG PROVINCE

HELEN JOSEPH HOSPITAL
TB FOCAL POINT
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Right to care
TREATING AIDS SERIOUSLY

24 May 2012

To: Dr S.A. van Blydenstein

Dear Dr van Blydenstein,

Herewith I would like to provide you with written permission to undertake the research project "**A DESCRIPTIVE STUDY OF RESISTANT TUBERCULOSIS CASES DIAGNOSED AT TB FOCAL POINT, HELEN JOSEPH HOSPITAL**" at the TB focal point, Helen Joseph Hospital after ethics approval is obtained.

Kind regards

Dr Eefje Jong
Medical Manager

Sr Josephine Tsotetsi
Nurse Manager

Clinical HIV Research Unit, Department of Medicine

Helen Joseph Hospital, Themba Lethu Clinic, Perth Road, Westdene, Johannesburg 2092, South Africa
Postnet Suite 176, Private Bag X2600, Houghton 2041, South Africa • Tel: +27 11 276-8800 • Fax: +27 11 482-2130

27 March 2012

To: Ms. S.A. van Blydenstein
Registrar Internal Medicine
Dept Internal Medicine
University of Witwatersrand

Re: Letter of permission to perform study at research site

Dear Ms. Van Blydenstein,

With this letter I wish to grant you permission to conduct your study entitled "A descriptive study of mono- and polyresistant tuberculosis cases diagnosed at TB focal point, Helen Joseph Hospital" in fulfillment of your MMed Internal Medicine at our site.

Please send me a copy of the ethics approval once obtained.

Kind regards,

Prof I.M. Sanne
Clinical Director Clinical HIV Research Unit
Department of Medicine
University of the Witwatersrand
Themba Lethu Clinic, Helen Joseph Hospital
Perth Ave, Westdene
South Africa
Tel no: 011 276 8800
Fax no: 011 482 2130
E-mail: isanne@witshealth.co.za